Supplementary Material

Figure S1. A-C. Flow cytometry analysis of cell surface uPA and uPAR expression on PDAC, wild-type and SerpinB2$^{-/-}$ MEFs; Expression of cell surface uPA and uPAR was analysed by indirect immunofluorescence using dual color flow cytometry as previously described (Andronicos and Ranson, 2001). Data was analyzed using FlowJo software version 7.6.5 (FlowJO LLC, USA) comparing specific antibody binding to isotype controls to account for nonspecific binding. Antibody details and dilutions are described in Table S1. **D**. Western blots showing expression of SerpinB2 in PDACs, SerpinB2$^{-/-}$ and wild-type MEFs. Whole cell lysates were subjected to non-reducing 10% SDS-PAGE (20 – 30 µg/lane) and resolved proteins transferred onto polyvinylidene fluoride membranes prior to blocking with 10% skim milk and enhanced chemiluminescence-based detection of antibody complexes. Blots were reprobed for actin as a loading control.

Figure S2. aSMA, uPA, uPAR and SerpinB2 antigen distribution in mixed cell allografts. **A, B.** Immunofluorescence imaging of cryosections from wild-type or SerpinB2$^{-/-}$ MEF:PDAC tumor allografts showing localization of alpha-SMA, uPA, uPAR (A) or alpha-SMA, SerpinB2, uPAR (B). Merged images include DAPI nuclear staining; **C.** Immunofluorescence imaging of cryosections from wild-type or SerpinB2$^{-/-}$ MEF:PDAC tumor allografts stained with IgG_1 and IgG_{2A} isotype controls. Merged images include DAPI nuclear staining.

Figure S3. Representative photomicrograph images defining quantification system of local invasion by either wild-type or SerpinB2$^{-/-}$ MEF:PDAC tumors. Cell invasion into local tissue (subcutaneous fat, muscle and skin) was analysed by histoscore on 4µm H&E stained sections. No invasion was quantified as 0; single cell invasion into local tissue was scored as 1, collective cell invasion as 2, and in the event where the tissue was completely penetrated

by tumor, a score of 3 was given. Quantification of local invasion was scored blind by three separate researchers.

Figure S4. Collagen I matrix contraction using independently derived MEFs. A. Photographs showing collagen I matrix contraction over 12 days in the presence of either wild-type or SerpinB2$^{-/-}$ MEFs **B.** Changes in area (mm^2) of collagen matrices shown in (**A**) over the 12 day contraction period. **C-D:** Maximum projection through 0 – 80 µm z-stack of SHG signal intensity of collagen I matrices formed with either wild-type or SerpinB2$^{-/-}$ MEFs **D.** Quantification of SHG signal intensity within matrices formed by either wild-type or SerpinB2$^{-/-}$ MEFs, inset: Mean SHG signal peak. Values shown are means ± SEM from 3 separate experiments, statistical analysis performed using an unpaired t-test.

Figure S5. Case-to-case matched analysis of PLAU, PLAUR and SERPINB2 mRNA expression in the APGI pancreatic cancer cohort. mRNA expression profiles of 142 PDAC cases were analysed using Illumina human HT-12 arrays (V4) as previously described (6). Data is available through GEO (accession GSE36924) and in the ICGC Data Coordination Centre (http://dcc.icgc.org/). Labels show gene and probe ID.

Table S1. Antibodies used for immunohistochemistry, immunofluorescence and western blotting.

Supplementary Movie Legends

Movie S1. Animated z-stack of SHG imaging from wild-type MEF contracted matrices at Day 4 of contraction.

Movie S2. Animated z-stack of SHG imaging from wild-type MEF contracted matrices at Day 12 of contraction.

Movie S3. Animated z-stack of SHG imaging from SerpinB2$^{-/-}$ MEF contracted matrices at Day 4 of contraction.

Movie S4. Animated z-stack of SHG imaging from SerpinB2$^{-/-}$ MEF contracted matrices at Day 12 of contraction.

Movie S5. Animation of rendered z-stacks of SHG imaging from SerpinB2$^{-/-}$ (L) and wild-type (R) MEF contracted matrices at Day 12 of contraction.

Movie S6 and S7. Animation of Migration of wild-type (S6) or SerpinB2$^{-/-}$ (S7) MEFs through Collagen I matrices over 10 h at the midpoint of matrix contraction (day 6). Collagen (magenta) was detected using SHG and wild-type or SerpinB2$^{-/-}$ MEFs were detected through stable GFP expression.

Figure S1 - Harris et al

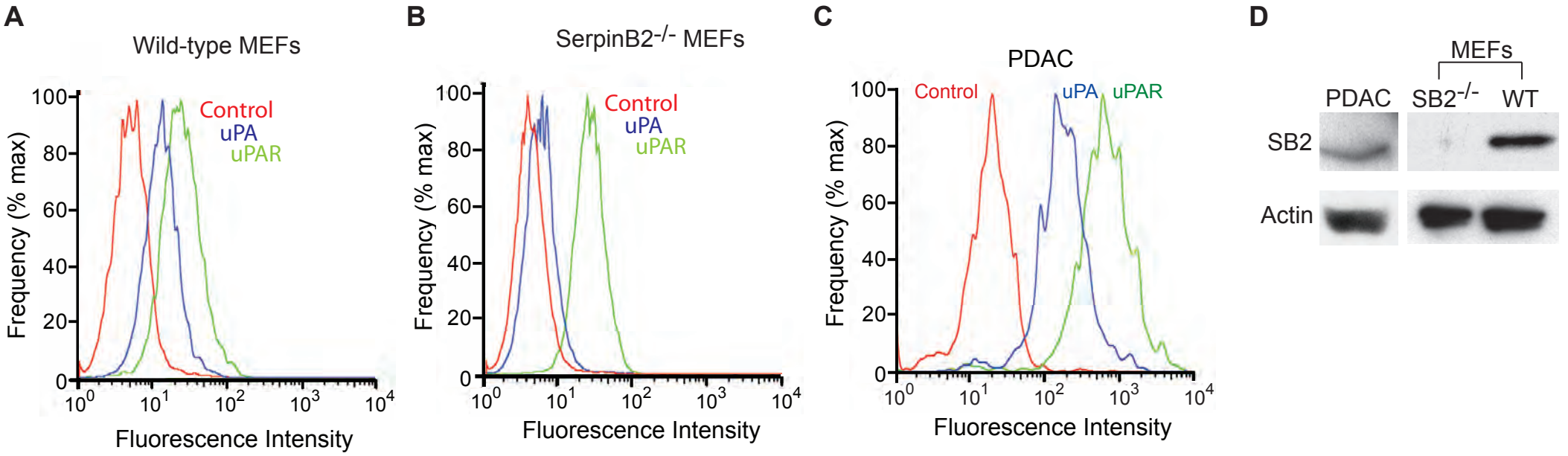


Figure S2 - Harris et al.

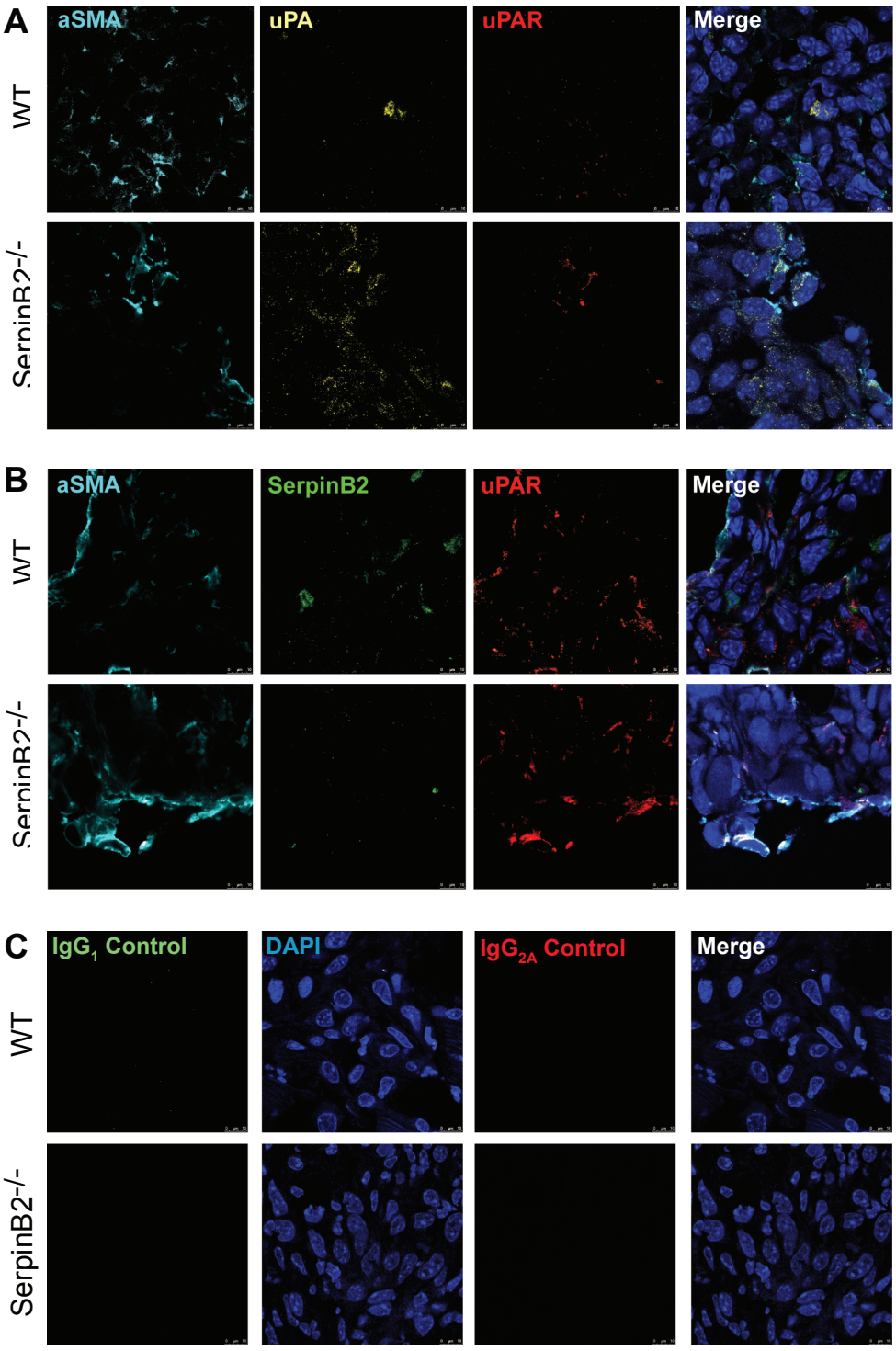
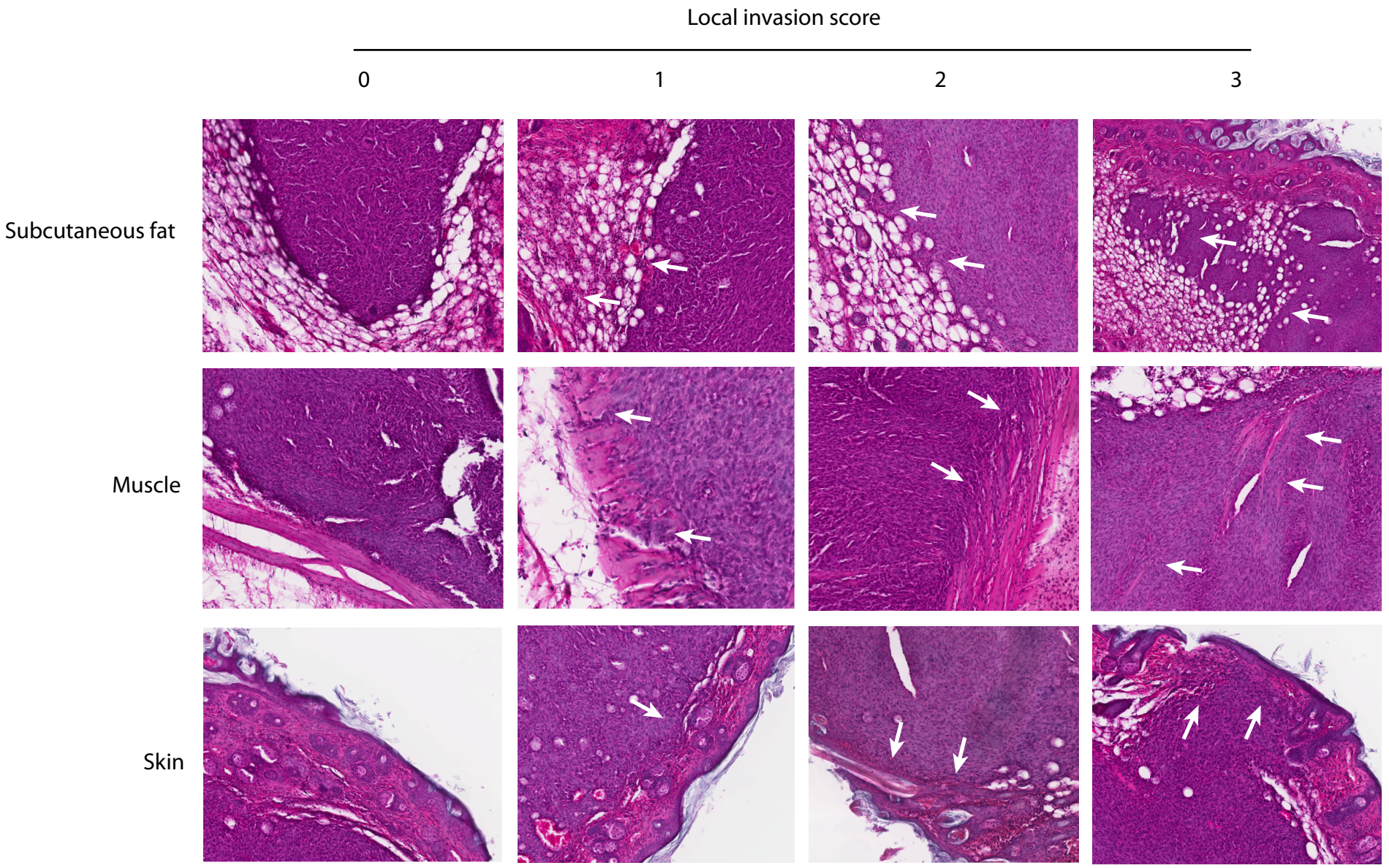
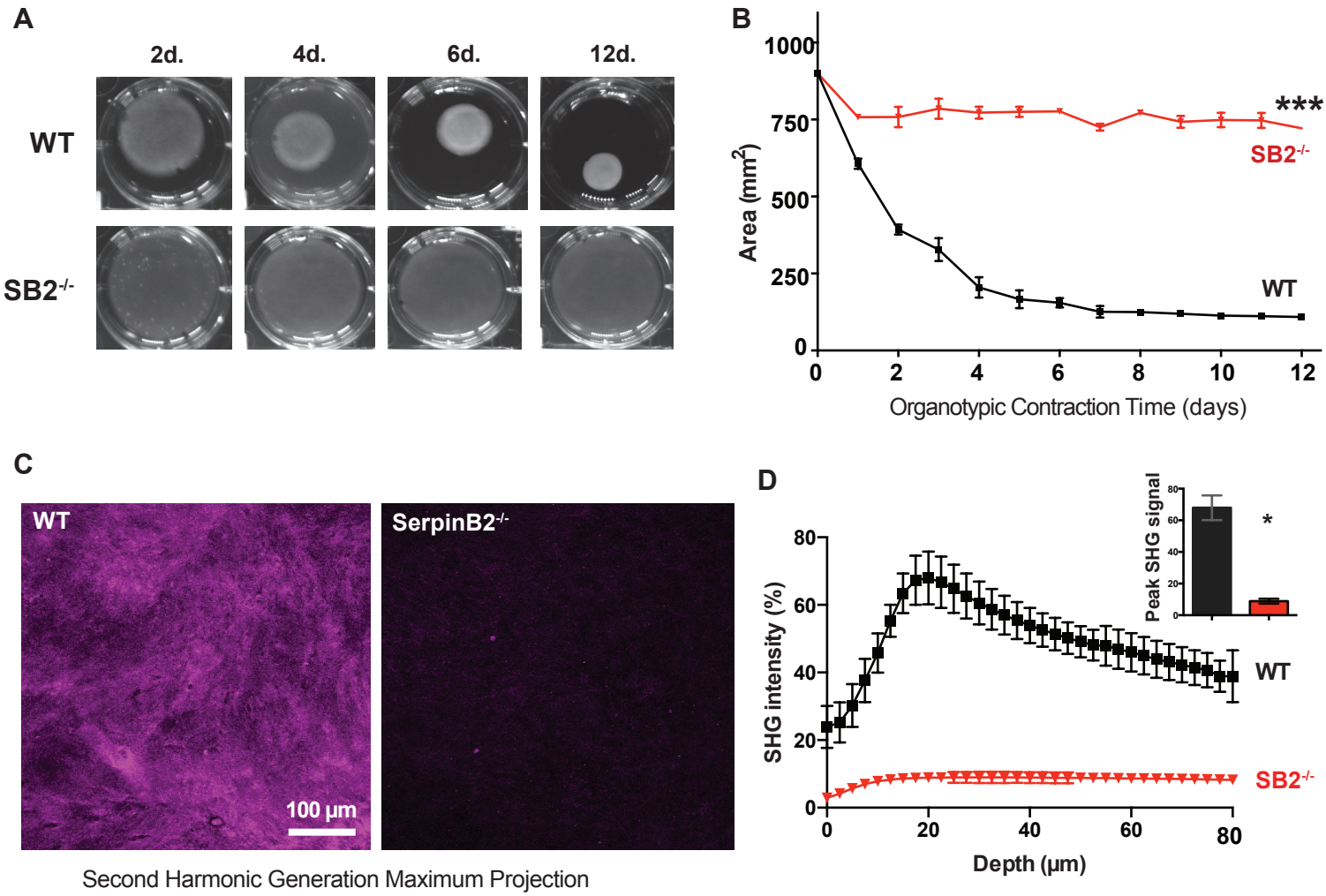


Figure S3 - Harris et al.



Harris et al - Fig S4



Harris et al - Fig S5

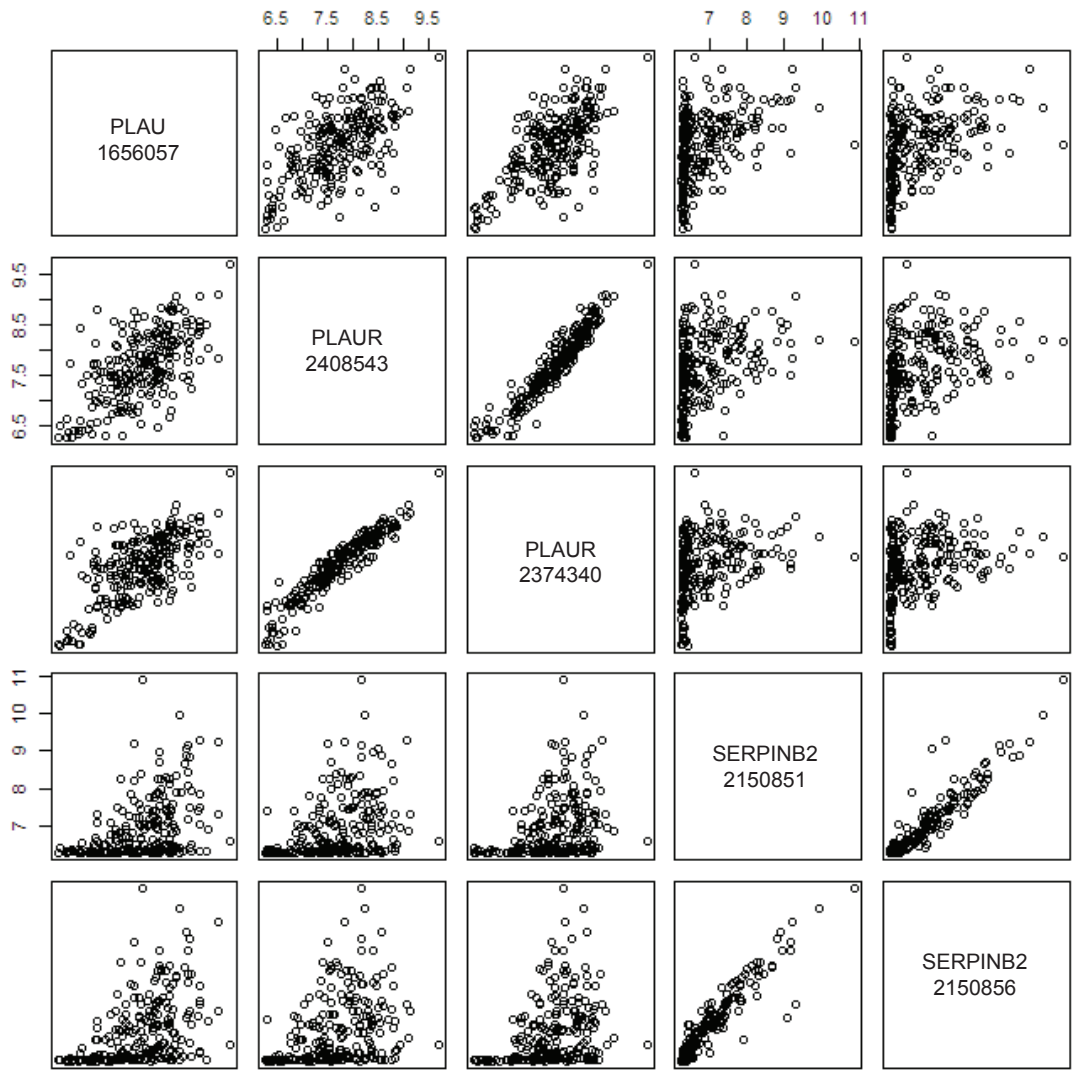


Table S1. List of primary antibodies used for immunofluorescence (IF), immunohistochemistry (IHC) and western blotting (WB) analyses

Antigen	Antibody description	Antibody supplier & catalog #	Antibody dilutions used	Secondary antibodies[1]
Actin	Mouse monoclonal, recognises multiple species (IgG_1)	Abcam; ab3280	IF: N/A IHC: N/A WB: 1:2500	Anti-mouse IgG-HRP[2]
Murine α-smooth muscle actin	Mouse monoclonal, cross-reacts with human (IgG_{2A})	Abcam; ab7817	IF: 1:500 IHC: N/A WB: N/A	Anti-mouse IgG Alexa Fluor 647
Chicken IgG	Mouse monoclonal (IgG_1)	Merck Millipore; MABC002	Negative control antibody - diluted to the same concentration as the test antibodies	
Human cleaved caspase-3	Rabbit polyclonal, cross-reacts with mouse (IgG)	Cell Signaling; #9661	IF: N/A IHC: 1:300 WB: N/A	Goat–anti rabbit IgG-HRP
Ki67	Rabbit monoclonal (IgG)	Thermo Scientific; RM9106-S1	IF: N/A IHC: 1:500 WB: N/A	Goat–anti rabbit IgG-HRP
Murine Multi-Cytokeratin	Mouse monoclonal (IgG_1)	Novocastra; NCL-C11	IF: N/A IHC: 1:500 WB: N/A	Goat–anti mouse IgG-HRP
Naïve rabbit sera Rabbit	Rabbit polyclonal (IgG)	Various	Negative control antibody - diluted to the same concentration as the test antibodies	
Murine SerpinE1	Rabbit Polyclonal (IgG)	Abcam; ab28207	IF: 1:500	Goat anti-rabbit Alexa-Fluor 488
Murine/human SerpinB2	Rabbit polyclonal (IgG)	Abcam; ab137588	IF: 1:500 IHC: N/A WB: 1:2000	Goat anti-rabbit Alexa-Fluor 488 Anti-rabbit IgG –HRP
Murine SerpinB2	Rabbit polyclonal (IgG) (raised against the CD loop)	(1)	IF: 1:500 IHC: N/A WB: 1:2000	Goat anti-rabbit Alexa Fluor 488 Anti-rabbit IgG –HRP
Trinitrophenyl–KLH	Rat monoclonal (IgG_{2A})	BD Pharmingen; 553996	Negative control antibody - diluted to the same concentration as the test antibodies	
Human uPA	Mouse monoclonal (IgG_1)	Sekisui Diagnostics GmbH: ADG3689	IF: 1:250 IHC: N/A WB: N/A	Goat anti-mouse IgG-FITC
Murine uPA	Rabbit polyclonal (IgG)	Abcam; ab20789	IF: 1:500 IHC: N/A WB: N/A	Goat anti-rabbit Alexa Fluor 488

[1] For flow cytometry, IgG-FITC secondary antibodies were from Sigma-Aldrich. For IF, IgG-Alexa Fluor secondary antibodies were from Life Technologies. For IHC and western blotting, IgG-HRP secondary antibodies were from DAKO and Sigma-Aldrich, respectively. Secondary antibodies were used within concentration ranges recommended by the manufacturers.

[2] Bound secondary antibodies detected using PicoWest ECL reagent (Pierce) and autoradiography

Table S1 – Harris et al

Human uPAR	Mouse monoclonal (IgG_1)	R&D Systems; MAB807	IF: 1:500 IHC: N/A WB: N/A	Goat anti-mouse IgG-FITC
Murine uPAR	Rat monoclonal (IgG_{2A})	R&D Systems; MAB531	IF: 1:500 IHC: N/A WB: N/A	Goat anti-rat IgG-FITC or IgG-Alexa Fluor 555
Murine IgG	Negative Control Mouse monoclonal (IgG_1)	DAKO; X0943	Negative control antibody - diluted to the same concentration as the test antibodies	
Rabbit IgG	Rabbit polyclonal	DAKO; X0903	Negative control antibody - diluted to the same concentration as the test antibodies	

1. Lee JA, Yerbury JJ, Farrawell N, Shearer RF, Constantinescu P, Hatters DM, et al. SerpinB2 (PAI-2) Modulates Proteostasis via Binding Misfolded Proteins and Promotion of Cytoprotective Inclusion Formation. Kampinga HH, editor. PLoS ONE. Public Library of Science; 2015;10:e0130136.